Tumor Necrosis Factor-α −308 Polymorphism and Leg Ulceration – Possible Association with Obesity

Journal of Investigative Dermatology (2007) **127,** 1768–1769; doi:10.1038/sj.jid.5700769; published online 22 March 2007

TO THE EDITOR

Polymorphism–disease association studies have been of increasing importance in the discovery of genetic backgrounds of multifactorial disorders; their value can be further increased by independent multiple investigations in geographically and ethnically distant populations. This commentary letter refers to an article by Wallace *et al.* (2006), recently published in this journal. Wallace *et al.* found a higher incidence of the A allele of −308 G/A single nucleotide polymorphism (SNP) located in the promoter region of the tumor necrosis factor-α (TNFα) gene among venous leg ulcer patients than in healthy controls.

TNFα is a potent pleiotropic proinflammatory cytokine with effects on lipid metabolism, coagulation, insulin resistance, and endothelial functions. It mediates several biological processes including wound healing (Crist *et al.*, 2004; Babbar *et al.*, 2006). It has also been demonstrated that TNFα is downregulated in wound fluid from nonhealing venous leg ulcers when compared with healing venous leg ulcers (Wallace and Stacey, 1998).

Venous leg ulcer is a multifactorial disorder; both genetic and environmental factors play essential roles in its pathogenesis; however, the pathomechanism of the disease is still not fully understood. Therefore, detecting SNPs, which might play a role in the development of venous leg ulcer, could result in identifying genetic factors leading to leg ulcer susceptibility (Nagy *et al.*, 2005). The −308 G/A polymorphism of the TNFα gene reported by Wallace *et al.* (2006) is the first TNFα SNP associated with wound healing and susceptibility for venous leg ulcer. The −308 TNFα SNP has been intensively studied previously. Among many other human diseases (Cancello *et al.*, 2004), its A allele has been linked to obesity and insulin resistance as well (Hoffstedt *et al.*, 2000; Brand *et al.*, 2001); however, others could not prove this connection (Walston *et al.*, 1999).

This study compared the frequency of the −308 G/A TNFα SNP in venous leg ulcer patients ($n=151$) and in healthy controls ($n=92$) in the Hungarian population. Because the −308 polymorphism of TNFα has been linked to increased obesity risk, the group of leg ulcer patients was divided into two subgroups: leg ulcer patients with body mass index (BMI) $<25\,kg/m^2$ ($n=110$) and with BMI $>25\,kg/m^2$ ($n=41$). Leg ulcer patients with diabetes, both type 1 and type 2, were excluded from the study. Venous leg ulcer patients were also screened for trauma, erysipelas, cardiac diseases, atherosclerosis, and autoimmune disorders. A leg-ulcer-free control group was divided into two groups: with BMI $<25\,kg/m^2$ ($n=92$) and with BMI $>25\,kg/m^2$ ($n=39$). Informed consent, approved by the Internal Review Board, was obtained from all donors; the study was conducted according to the Declaration of Helsinki Principles.

Blood samples were taken from all participants ($n=282$), and DNA was isolated by a standard proteinase K digestion method (Eppendorf AG, Hamburg, Germany). The TNFα −308 G/A SNP was genotyped by the PCR-restriction-fragment length polymorphism (RFLP) method: 100 ng of genomic DNA was used for PCR amplification and a 147-nt long region of the TNFα promoter harboring the SNP was amplified by PCR using the following primers: forward 5′-gaggcaataggtttt gagggccat-3′, reverse 5′-gggacacacaag catcaag-3′. *Nco*I (Fermantas, Vilnius, Lithuania) restriction enzyme digestion was performed to distinguish the −308 G/A alleles using 10 μl of the PCR products, run on 5% Nusieve agarose gels (Cambrex, Berkshire, UK) and photographed. The amplified wild-type (GG) PCR product was cut at one position by *Nco*I, resulting in 121- and 26-nt long bands; the amplified homozygote mutant (AA) PCR product was not cut. Therefore the original 147-nt PCR product was detected, and the heterozygote mutant genotype was characterized by a pattern of 147, 121, and 26 nt products.

Statistical analysis was carried out on the groups of leg ulcer patients and controls according to the rules of case–control allelic association study design. The statistical significance of association between the −308 TNFα SNP and venous leg ulcer was assessed by the Fisher exact probability test, and odds ratios (ORs) with 95% confidence intervals (CIs) were also calculated (VassarStats, http://faculty.vassar.edu/lowry/VassarStats.html). To be comparable with the referred paper (Wallace *et al.*, 2006), logistic regression analysis was also carried out.

When we compared the data of the non-obese venous leg ulcer patients (BMI $<25\,kg/m^2$) with non-obese healthy controls (BMI $<25\,kg/m^2$), we could not detect a difference in the mutant (A) allele frequency of the −308 TNFα SNP. However, the mutant allele frequency of the −308 TNFα SNP showed a significant difference (Fisher exact probability test $P=0.0273$, OR (95% CI) $=1.88$ (0.9533–3.6877)): simple logistic regression analysis $P=0.0514$) between obese venous leg ulcer patients

Abbreviations: BMI, body mass index; CI, confidence interval; OR, Odds ratio; SNP, single nucleotide polymorphism; TNFα, tumor necrosis factor-α

Table 1. PCR-RFLP data of the −308 G/A polymorphism of the TNFα gene

	GG		GA		AA						
TNFα gene −308 G/A SNP	M^2	F^2	M	F	M	F	G	A	Fisher's[1] test	Odds ratio	95% CI of OR
Leg ulcer patients with BMI > 25 kg/m² (*n*=41) (group 1)	25 (60.98)		14 (34.15)		2 (4.88)		64	18	*P*=0.0273	1.88	0.9533–3.6877
	9	16	6	8	1	1					
Leg ulcer patients with BMI < 25 kg/m² (*n*=110) (group 2)	76 (69.09)		31 (28.18)		3 (2.73)		183	37	*P*=0.0643	1.35	0.7732–2.3498
	31	45	12	19	1	2					
All leg ulcer patients (*n*=151) (group 1+2)	101 (66.89)		45 (29.80)		5 (3.31)		247	55	*P*=0.0334	1.49	0.8834–2.4948
	40	61	18	27	2	3					
Leg-ulcer-free controls with BMI > 25 kg/m² (*n*=39) (group 3)	24 (61.54)		14 (35.90)		1 (2.56)		62	16	*P*=0.0457	1.72	0.8567–3.4549
	0	24	0	14	0	1					
Leg-ulcer-free controls with BMI < 25 kg/m² (*n*=92) (group 4)	68 (73.91)		24 (26.09)		0		160	24			
	29	39	11	13	0	0					
All leg-ulcer-free controls (*n*=131) (group 3+4)	92 (70.23)		38 (29.01)		1 (0.76)		222	40	*P*=0.0888	1.20	0.6963–2.0724
	29	63	11	27	0	1					

BMI, body mass index; RFLP, restriction-fragment length polymorphism; TNFα, tumor necrosis factor-α.
Data in parentheses show numbers of individuals with percentages.
[1]Fisher's exact probability test was calculated using allele frequency data of the A and G alleles.
[2]M, number of males; F, number of females.

(BMI > 25 kg/m²) and non-obese healthy controls (BMI < 25 kg/m²) (Table 1). We also compared the genotype data of the −308 TNFα SNP of the unified group of leg ulcer patients without considering their BMI with healthy controls, but there was no difference. Our data are in agreement with the findings of Hoffstedt *et al.* (2000) and Brand *et al.* (2001); we found a higher frequency of A allele of TNFα −308 SNP in the obese control group compared with the non-obese control group (Fisher exact probability test $P = 0.0457$, OR (95% CI) = 1.72 (0.8567–3.4549)).

According to our data, the level of association between −308 TNFα SNP and venous leg ulcer susceptibility might depend on the BMI of the Hungarian individuals enrolled in the study. As obesity was not considered in the Wallace paper, there is a possibility that the association between the A allele of the −308 TNFα SNP and leg ulcer susceptibility they found was because of the suspected high rate of obese individuals in the Australian leg ulcer patients' group. Thus, our results are in agreement with the conclusion of Wallace *et al.* (2006) that the A allele of the −308 TNFα SNP might be a potential factor for venous leg ulcer susceptibility; however, our data suggest that this association is secondary and the primary association probably exists with obesity.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by grants NKFP1A/0012/2002, OTKA TS044826, NI62007 and T 042455, GVOP-3.2.2-2004-07-0010/3.1, GVOP-3.2.1-2004-04-0372/3.0, and GVOP-3.2.1-2004-040372/3.0, and ETT 530/2006. Márta Széll was supported by the Bolyai Foundation of the Hungarian Academy of Sciences.

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